Supporting Information

Dynorphin neuropeptides decrease apparent proton affinity of ASIC1a by occluding the acidic pocket

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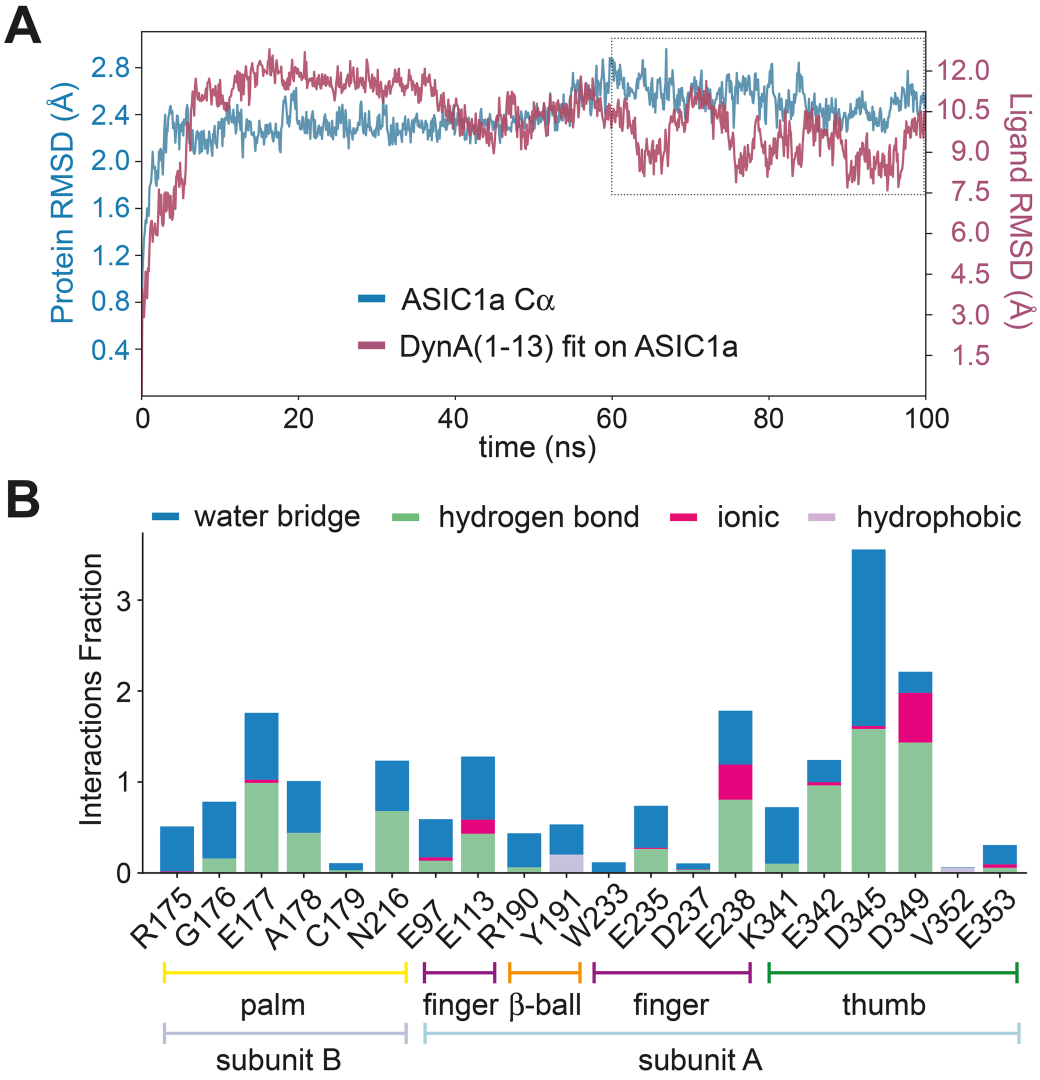


Figure S1. Molecular dynamics simulation of the Dyn A(1-13)-ASIC1a interaction. (A) Root mean square deviation (RMSD) of the protein Cα atoms and the ligand with respect to the initial structure. **(B)** Histogram showing the number of non-covalent interactions observed over 95% of the simulation time (60-100 ns), color-coded by their particular type. Water bridges, hydrogen bonds and ionic interactions are electrostatic interactions. The stacked bar charts are normalized over the course of the trajectory: for example, a value of 0.5 suggests that 50% of the simulation time the specific interaction is maintained. Values >1.0 are possible as some protein residue may make multiple contacts of the same subtype with the ligand. The domains of two ASIC1a subunits that mediate the interaction are indicated.

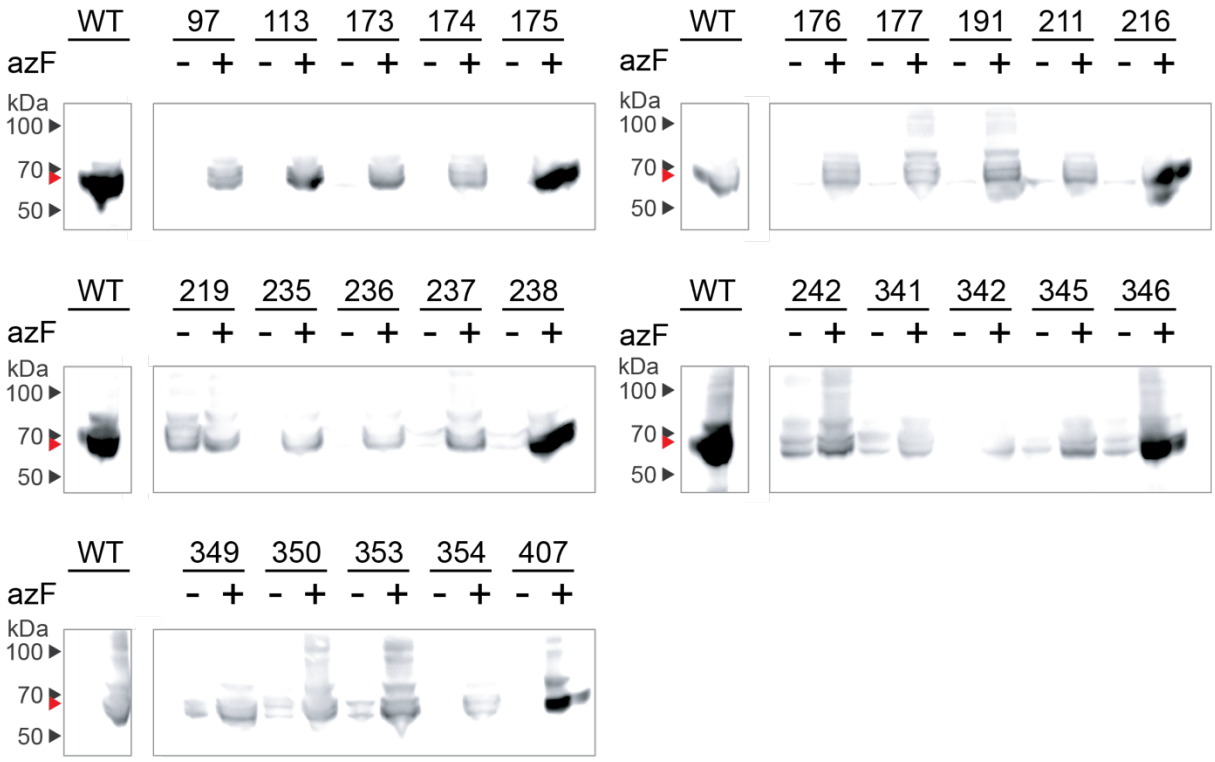


Figure S2. Site-specific incorporation of azF into ASIC1a is efficient. All 25 ASIC1a mutants have been expressed in the absence (-) or presence (+) of 0.5 mM azF in the cell media. About 40 h after transfection, cells were lysed directly in Laemmli sample buffer and subjected to a western blot procedure. Expression of ASIC1a constructs was detected with an anti-ASIC1a antibody. *Red arrow heads* point to ASIC1a full-length monomers. At all positions (besides 219) azF was incorporated efficiently (high signal for '+ azF' conditions, no or low signal for '- azF' conditions). At position 219, non-specific incorporation of canonical amino acids was interfering with azF incorporation. These experiments have been performed twice independently.

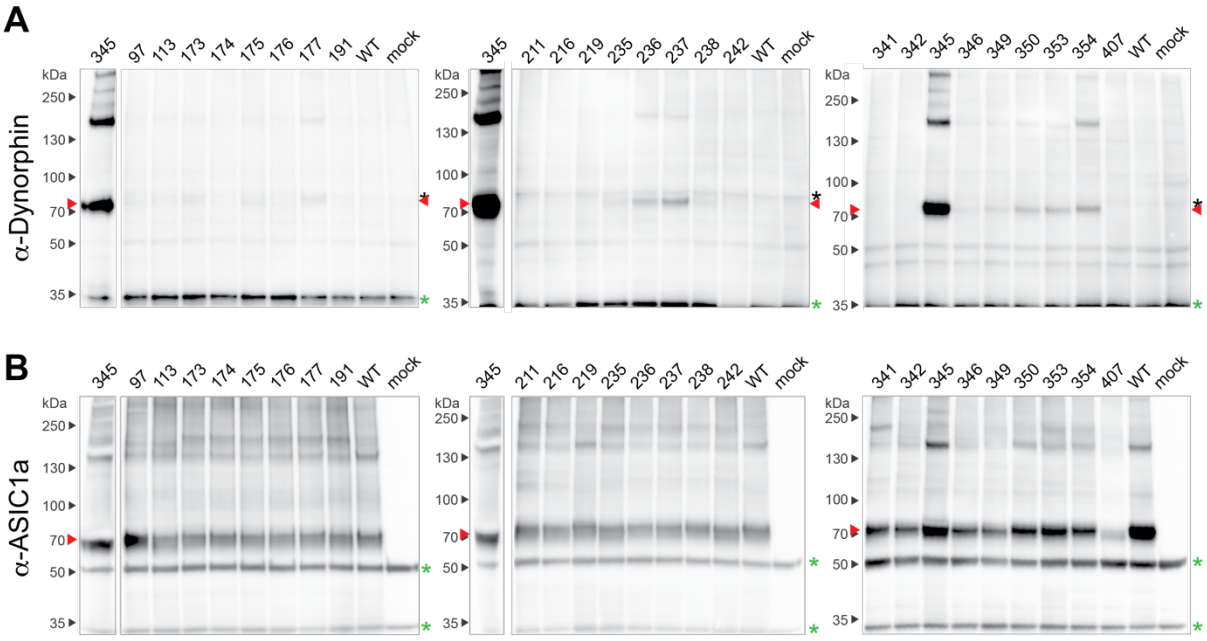


Figure S3. Detection of Big Dyn and ASIC1a after photo-crosslinking and immunoprecipitation. The same blots as in Figure 2B are shown here in full size. After detection of Big Dyn (A), the blots have been stripped and developed for ASIC1a (B). *Red arrow heads* point to covalent ASIC1a-Big Dyn complexes (A) and to monomeric ASIC1a (B), respectively. In (A), *black stars* indicate non-specific bands that run close to the specific bands (i.e. covalent ASIC1a-Big Dyn complexes). G*reen stars* show the signal that emerged from antibodies used for the immunoprecipitation of ASIC1a. Bands in the upper molecular weight range (>130 kDa) may indicate ASIC1a multimers.

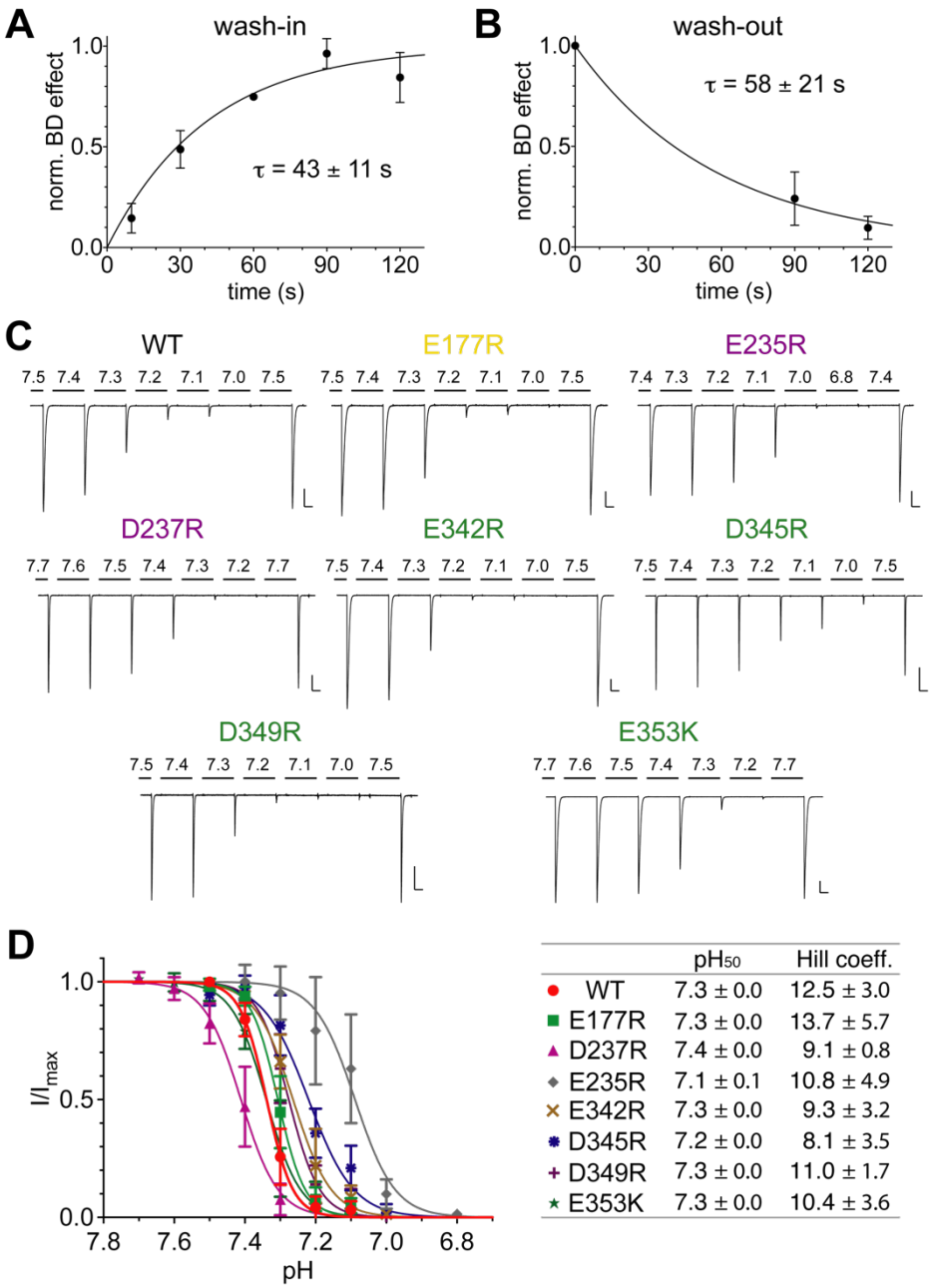


Figure S4. Functional analysis of ASIC1a WT and mutants. (A, B) Wash-in (A) and wash-out (B) times for the modulation by Big Dyn (BD). Pre-conditioning pH of 7.15 was used which resulted in a maximal Big Dyn effect of 42 ± 4% of I_{max} after ~90 s of washing in 2.5 μM Big Dyn. For wash-out experiments, Big Dyn was applied for 120 s at pH 7.15 and then washed out for varying times at pH 7.15. Fitting the kinetics yielded a time constant τ = 43 ± 11 s for wash-in (*n* = 4 for each time point) and τ = 58 ± 11 s for wash-out (*n* = 3-4 for each time point). Due to few time points, τ for wash-out is only a rough estimate. ASIC1a was activated with pH 6 for 10 s. **(C)** Representative TEVC recordings for SSD of ASIC1a WT and all mutants. *Horizontal scale bars*, 20 s; *vertical scale bars*, 2 μA. **(D)** SSD curves for ASIC1a WT and mutants are shown on the left, corresponding pH_{50} and Hill coefficient values obtained from fits are shown on the right (*n* = 5-8). In (A-B) and (D), data are shown as mean ± SD.

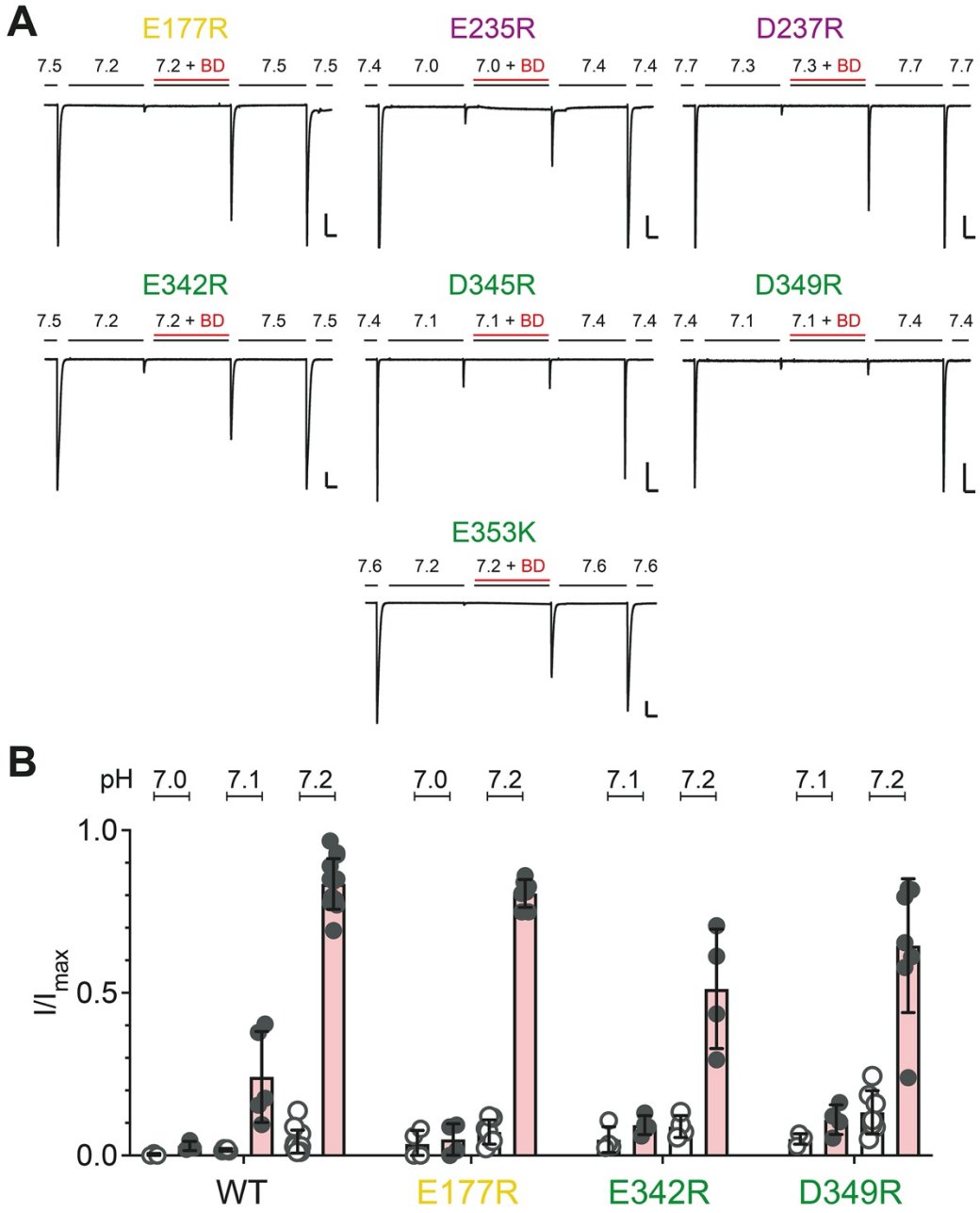
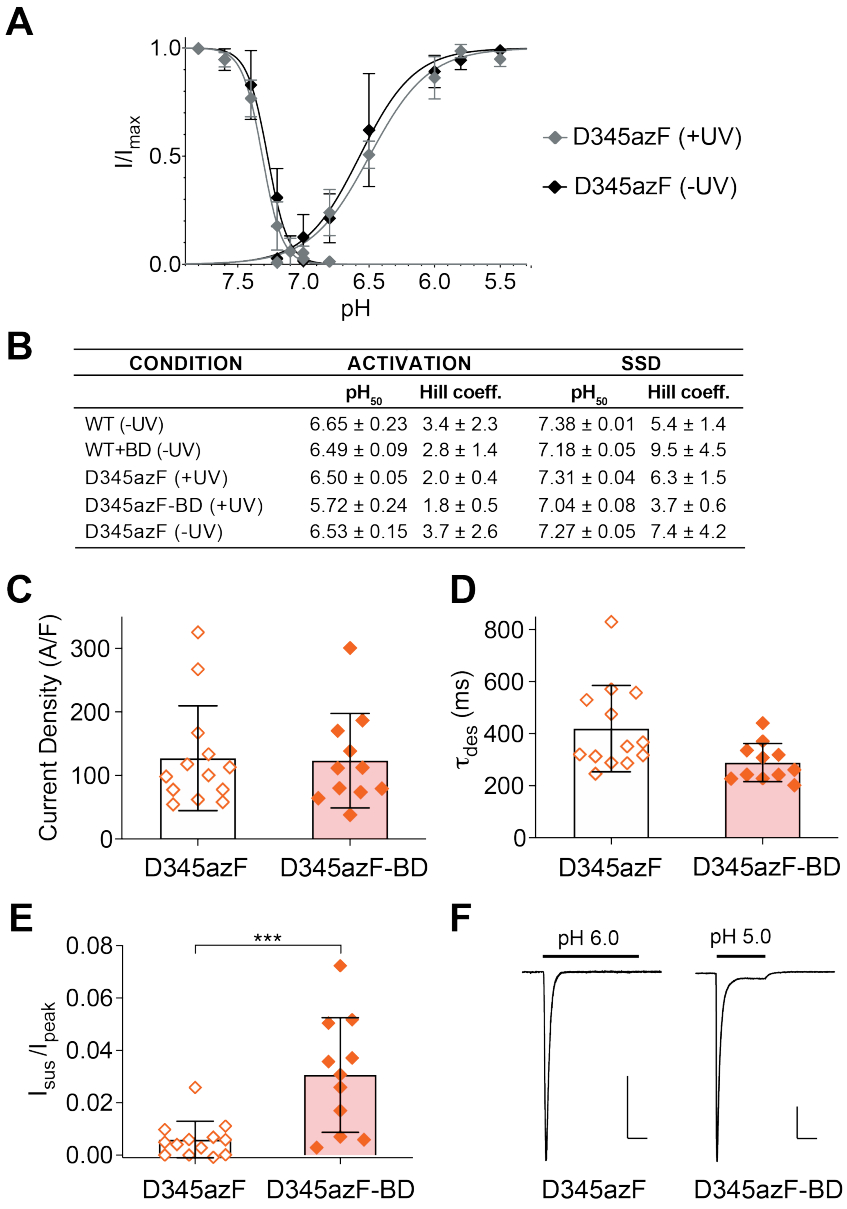


Figure S5. Conditioning pH determines the extent of ASIC1a modulation by Big Dyn. (A) Representative TEVC recordings for ASIC1a mutants shown in Figure 3C. *Horizontal scale bars*, 20 s; *vertical scale bars*, 2 μA. **(B)** For three mutants and the WT, the Big Dyn (BD) effect was determined at different pre-conditioning pH solutions. Small reductions in pre-conditioning pH, e.g. from 7.2 to 7.1, had only marginal effects on desensitized current amplitudes but drastically decreased the Big Dyn effect. Shown are relative current amplitudes elicited after pre-conditioning in the absence (*empty circles, white bars*) and presence of 2.5 μM Big Dyn (*filled circles, red bars*). Bar graphs are dot plots with mean ± SD; *n* = 3-13 per condition.



CONDITION	ACTIVATION		SSD	
	pH_{50}	Hill coeff.	pH_{50}	Hill coeff.
WT (-UV)	6.65 ± 0.23	3.4 ± 2.3	7.38 ± 0.01	5.4 ± 1.4
WT+BD (-UV)	6.49 ± 0.09	2.8 ± 1.4	7.18 ± 0.05	9.5 ± 4.5
D345azF (+UV)	6.50 ± 0.05	2.0 ± 0.4	7.31 ± 0.04	6.3 ± 1.5
D345azF-BD (+UV)	5.72 ± 0.24	1.8 ± 0.5	7.04 ± 0.08	3.7 ± 0.6
D345azF (-UV)	6.53 ± 0.15	3.7 ± 2.6	7.27 ± 0.05	7.4 ± 4.2

Figure S6. Functional properties of ASIC1a with a covalently attached Big Dyn. (A) For D345azF, UV treatment (without Big Dyn) had no effect on pH dependence of activation or SSD. *Grey*, with UV pre-treatment (*n* = 5); *black*, without UV pre-treatment (*n* = 5). **(B)** Values (mean ± SD) from the fits shown in Figure 4C, D and (A). **(C, D)** At maximal activation (pH 6.0 for D345azF, pH 5.0 for D345azF-BD), neither the current density (C) nor the desensitization kinetics (D) were altered by the covalent link to Big Dyn. *n*(D345azF) = 13, *n*(D345azF-BD) = 11. **(E)** A small sustained current component (I_{sus}) was observed for D345azF-BD when maximally activating the channels (pH 6.0 for D345azF, pH 5.0 for D345azF-BD). *n*(D345azF) = 13, *n*(D345azF-BD) = 11; p = 0.0009. Bars represent mean ± SD. **(F)** Representative traces for recordings from which the data shown in (C-E) have been obtained. *Horizontal scale bars*, 2 s; *vertical scale bars*, 1 nA.

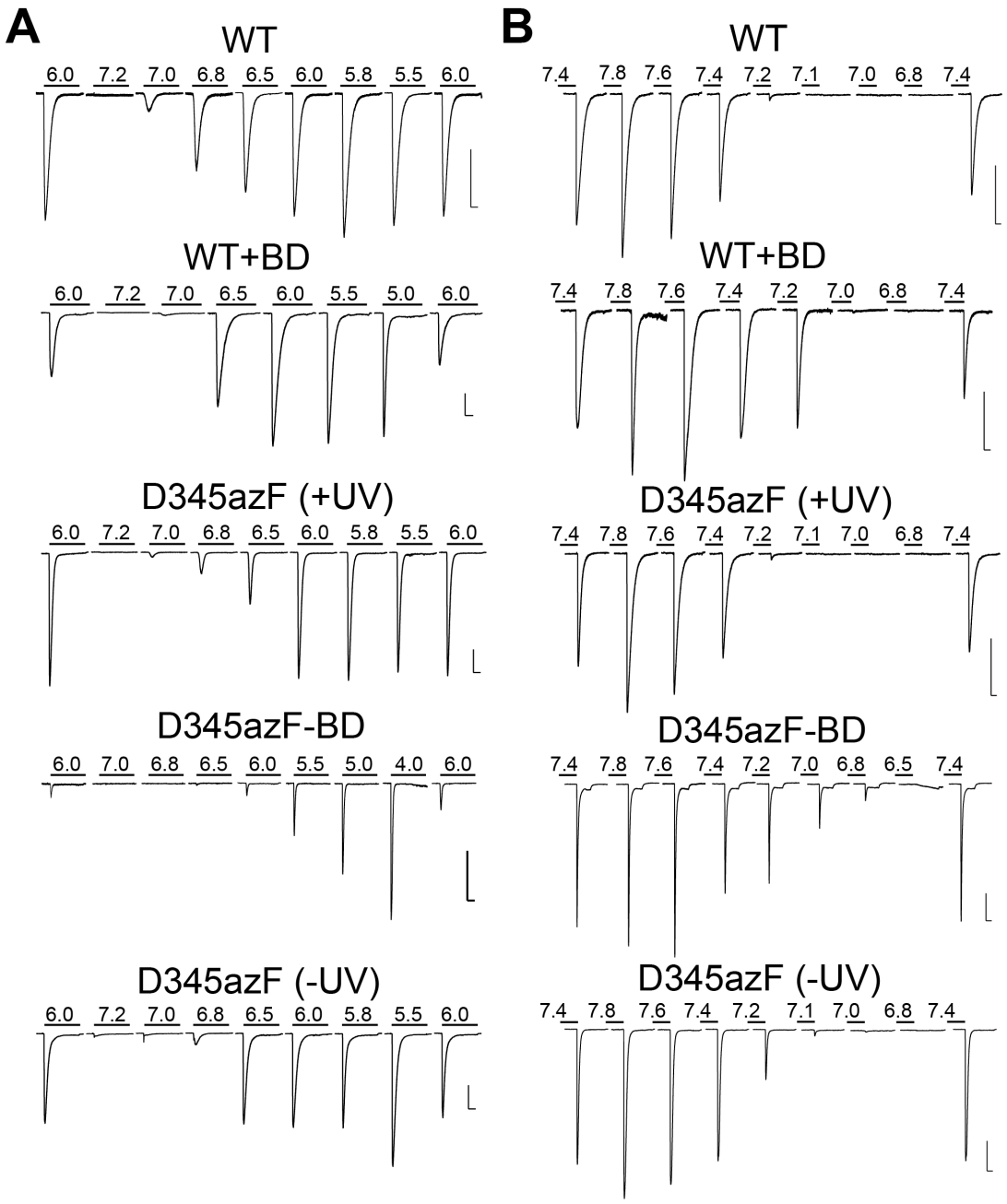


Figure S7. Representative patch clamp recordings of D345azF. (A) pH dependence of activation for all conditions presented in Figure 4C and Supporting Figure S6. pH values used for activation are indicated; pre-conditioning pH was 7.4. **(B)** pH dependence of SSD for all conditions presented in Figure 4C and Supporting Figure S6. Pre-conditioning pH values are indicated; activation was performed with pH 5.0 for D345azF-BD and with pH 6.0 for all other conditions. For the recordings of WT+BD and D345azF (+UV) shown here, the pre-conditioning pH solutions were applied in random order; the rest of the experiments has been performed in the order as illustrated. *Horizontal scale bars*, 2 s; *vertical scale bars*, 1 nA.

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CONDITION	ACTIVATION		SSD	
	pH_{50}	Hill coeff.	pH_{50}	Hill coeff.
D237azF (+UV)	6.43 ± 0.2	2.0 ± 0.1	7.34 ± 0.1	5.9 ± 0.6
D237azF-BD (+UV)	5.8 ± 0.08	1.3 ± 0.8	7.1 ± 0.01	4.5 ± 0.5
D237azF (-UV)	6.76 ± 0.01	2.5 ± 0.1	7.38 ± 0.02	6.8 ± 0.7

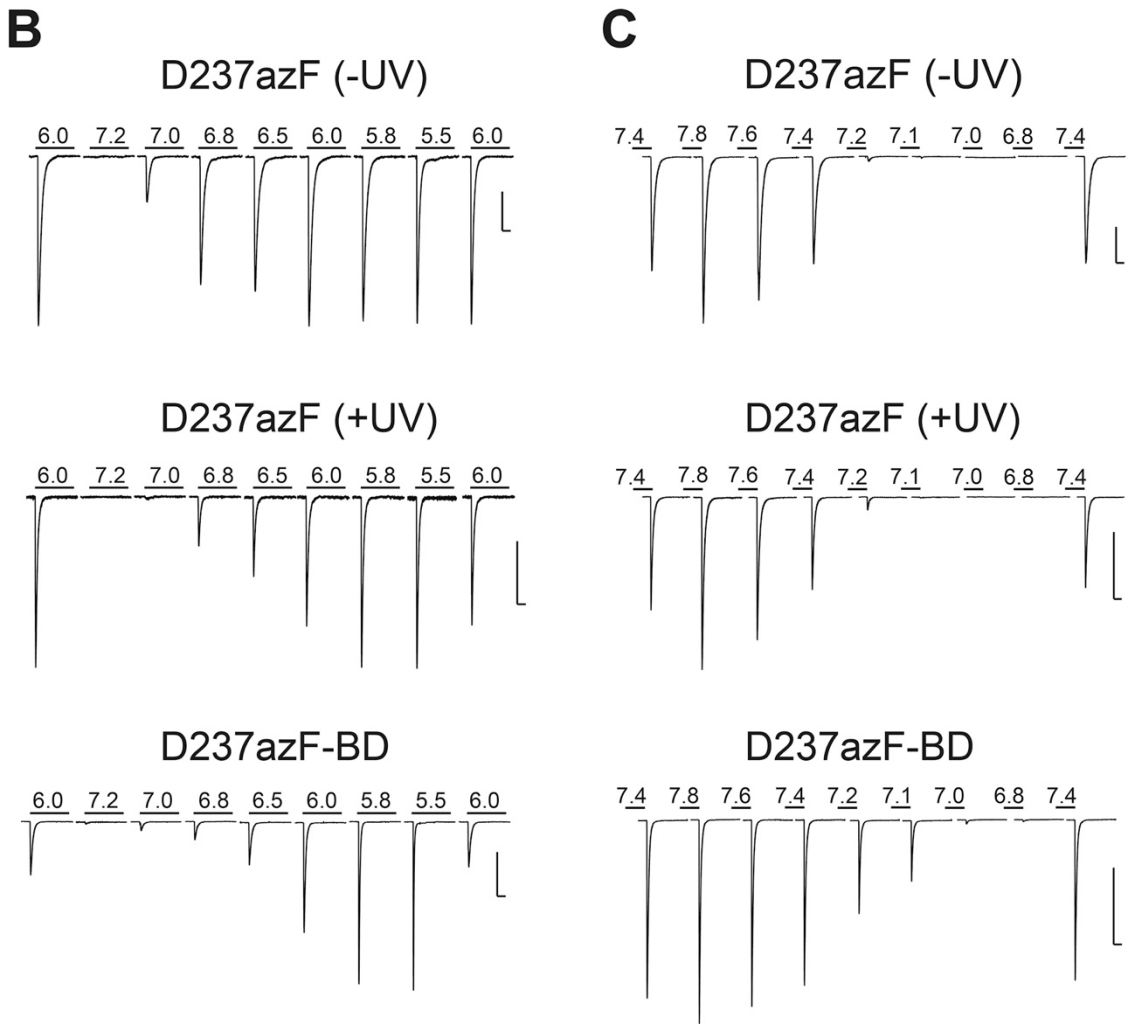

Figure S8. Representative patch clamp recordings of D237azF. (A) Values (mean ± SD) from the fits shown in Figure 5C. **(B)** pH dependence of activation for the three conditions presented in Figure 5C. pH values used for activation are indicated; pre-conditioning pH was 7.4. **(C)** pH dependence of SSD for the three conditions presented in Figure 5C. Pre-conditioning pH values are indicated; activation was performed with pH 5.0 for D237azF-BD and with pH 6.0 for the two other conditions. For all the recordings, the pre-conditioning pH solutions were applied in the order as illustrated. *Horizontal scale bars*, 2 s; *vertical scale bars*, 1 nA.

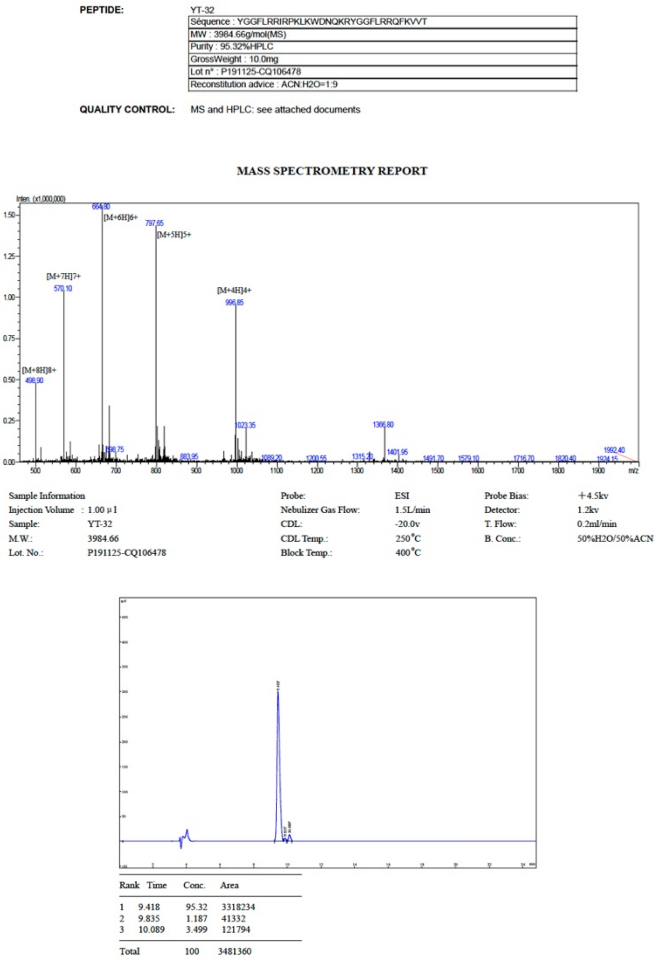

Figure S9. MS spectrum and HPLC chromatogram for Big Dyn. Big Dyn (YT-32) was purchased from ProteoGenix. Middle, MS spectrum. Bottom, HPLC chromatogram.